VEGETATIVE COMPATIBILITY AND GENETIC ANALYSIS OF *COLLETOTRICHUM LINDEMUTHIANUM* ISOLATES FROM BRAZIL

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INTRODUCTION

The causal agent of common bean anthracnose, *Colletotrichum lindemuthianum*, presents a wide genetic and pathogenic variability that causes complications in the development of resistant cultivars. The aim of this study was to identify the variability within and between Brazilian pathotypes of *C. lindemuthianum* through the identification of vegetative compatibility groups (VGCs) and by randomly amplified polymorphic DNA (RAPD) analysis.

MATERIALS AND METHODS

Forty seven *C. lindemuthianum* isolates belonging to 13 different pathotypes were collected from naturally infected bean cultivars produced in various regions of Brazil during the period 2000 to 2006. The method described by Brooker et al. (1991) was applied to the development of *nit* mutants. Following identification, mutants were characterised phenotypically and classified as *nit1*, *nit2*, *nit3* and *nitM* according to their growth parameters (Correll et al. 1987). The vegetative self-compatibilities of different *nit* mutants of a single isolate and the cross-compatibilities between *nit* mutants of different isolates were tested by pairing mycelia plugs and the dishes were incubated in the dark at 22°C for at least 4 weeks. The growth of aerial mycelia in the contact zone between the two colonies and the formation of heterokaryons were monitored weekly. For the RAPD analysis, DNA was extracted from the isolates according to a modified version of the method of Raeder and Broda (1985). A dendrogram was produced from the similarity matrix thus generated using the unweighted pair-group method with arithmetic means (UPGMA) with the assistance of NTSYS-PC 2.1 software (Rohlf 2000).

RESULTS AND DISCUSSION

A total of 295 *nit* mutants (279 *nit3*, 15 *nit1* and one *nitM*) were obtained from 47 isolates. In complementation tests, six of the isolates were shown to be heterokaryon self-incompatible, whilst the cross-complementation observed among *nit1* and *nit3* mutants of the different isolates enabled 45 VGCs to be identified. The high frequency of formation of *nit3* mutants and the phenomenon of self-incompatibility observed in the present study has already been described in other studies (Brooker et al. 1991; Beynon et al. 1995). A correlation between the presence of L-asparagine or L-threonine in MM + chlorate and the recovery of *nitM* mutants has been established by some researchers (Leslie and Summerell 2006), but in the present study supplementation of the medium with these amino acids did not increase the frequency of *mitM* mutants. In the molecular analyses, 18 RAPD primers were employed and 111 polymorphic bands obtained. The estimates of genetic similarities, determined from the Sorence-Dice coefficient, ranged from 0.42 to 0.97, and the dendrogram obtained by cluster analysis revealed 18 separate groups of isolates (Figure1). The intra- and inter-genetic variability within the *C. lindemuthianum* population established by RAPD analysis is in agreement with previous findings (Mahuku and Riascos 2004; Talamini et al. 2006; Damasceno e

Silva et al. 2007). In the present study, groups within *C. lindemuthianum* have been identified through the generation of mutant strains and by RAPD analysis and the results confirm the immense diversity exhibited by this species. The findings underline the difficulties incurred in achieving long-term resistance against anthracnose in the common bean and emphasise the need to elucidate the mechanisms responsible for the large pathogenic variability shown by this phytopathogen.

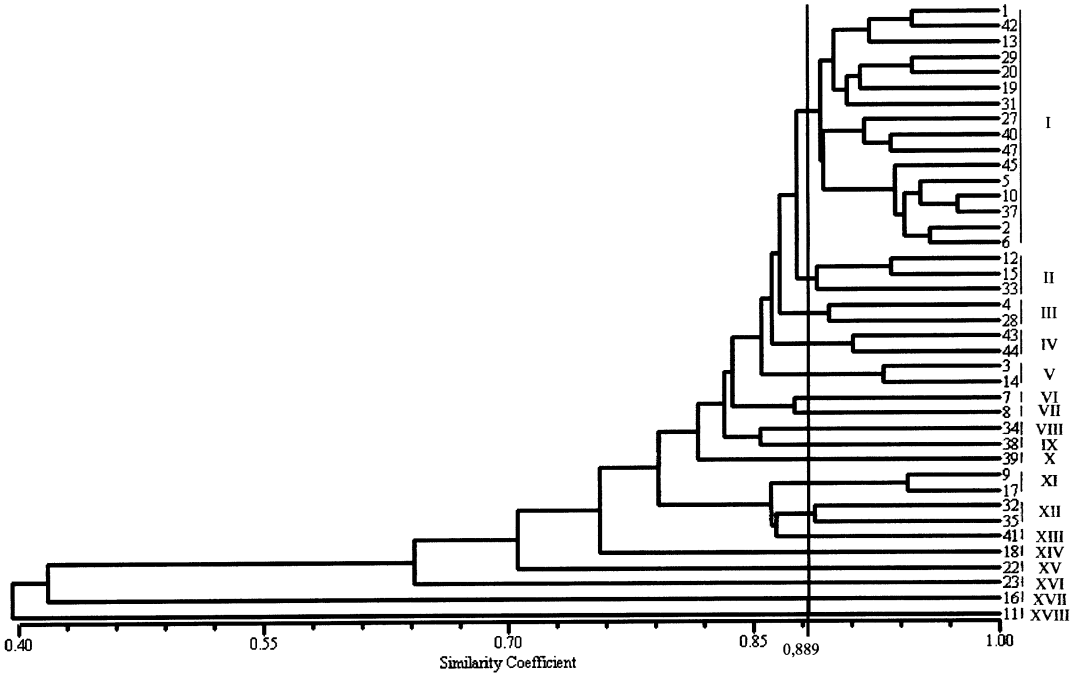

Fig. 1. Dendrogram showing the genetic similarity of isolates of *C. lindemuthianum* collected in different regions of Brazil.

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